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Radiological and Histological Variants of Thanatophoric Dysplasia Are Associated With Common Mutations in FGFR-3

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We describe two fetuses of the 21st week of gestation that share some macroscopic, radiologic, and histologic findings of thanatophoric dysplasia (TD), but also show distinct differences from the usual subtypes of TD. These differences mainly comprise the lack of facial abnormality, only mild reduction of chondrocyte proliferation and hypertrophy, and the lack of fibrous tissue interposition between cartilage and periosteal bone. Thus, these two cases may represent a distinct variant of thanatophoric dysplasia. The molecular analysis of the FGF-R-3 gene demonstrated in both cases mutations which were not significantly different from those of other cases of TD. Thus, the phenotypic modulation within the subtypes of TD may be influenced by additional and yet unknown factors.

KEY WORDS: bone dysplasia, thanatophoric dysplasia, FGFR-3 mutations

INTRODUCTION

Thanatophoric dysplasia (TD) is a lethal osteochondrodysplasia (OCD) first described by Maroteaux et al. in 1967. It occurs with an estimated frequency of about 0.3–0.5 in 10,000 births [Orioli et al., 1986; Martínez-Frías et al., 1988]. The main pathological findings comprise micromelia with symmetric shortness of the limbs along with relatively normal length of the trunk, severe frontal bossing with facial anomalies, and marked platyspondyly leading to a characteristic radiological pattern of the vertebral bodies [Maroteaux et al., 1967; Langer et al., 1969; Kaufman et al., 1970; Spranger and Maroteaux, 1990]. Histologically, a significant reduction of chondrocyte proliferation, poor organization of chondrocyte columns, and a typical interposition of fibrous tissue bands between cartilage and newly formed periosteal bone are usually visible [Horton et al., 1988; Spranger and Maroteaux, 1990]. These changes are related to the impaired cartilaginous growth which affects not only the long bones, but also the ribs. This in turn results in a narrow thorax and consequently hypoplastic lungs. Respiratory failure usually leads to death during or shortly after birth [Langer et al., 1969].

On the basis of radiological and clinical findings, two major subtypes have been delineated which basically differ in the length of the long bones and the extent of their bowing [Langer et al., 1987; Spranger and Maroteaux, 1990; Norman et al., 1992]. Thus, TD type I is referred to as having relatively short and curved femora, while TD type II shows straight and relatively long femora. TD type I may or may not be associated with cloverleaf skull deformity, while cases with TD type II mostly have a cloverleaf skull.

In addition to these two subtypes, other forms of lethal OCDs have been attributed to TD, e.g., the "Glasgow variant" [Spranger and Maroteaux, 1990], which differs from the other forms of TD by more regular inferior margins of the ilia, well-developed, round distal femoral epiphyses, and well-ossified vertebral bodies. Other variants have been also described, such as the "Oswestry variant" [Darby and Mercer, 1995] with unique histological abnormalities. Furthermore, there exist several lethal OCDs with severe platyspondyly, in the absence of the other TD changes. These disorders have been grouped into the "platyspondylic lethal OCDs" with several subtypes which have to be distinguished from TD [Spranger and Maroteaux, 1990].

Recent molecular biological analyses of TD cases have identified distinct mutations of the FGFR-3 (fibroblast growth factor receptor-3) gene in both common subtypes of TD [Tavormina et al., 1995; Rousseau et al., 1995]. In contrast, the molecular defects of the other platyspondylic lethal OCDs are not yet elucidated.

Here, we describe two fetuses with typical findings of TD, but unusual macroscopic and histological aspects

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Dedicated to Jürgen W. Spranger on the occasion of his 65th birthday with admiration and best wishes.

not usually seen in the two major subtypes of TD. The molecular analysis of DNA isolated from chondrocytes documented in both cases typical mutations of the FGFR-3 gene commonly seen in cases of "classical" TD type I. Our observations not only illustrate the clinical variability of lethal OCDs, such as TD, but also indicate that phenotypic modulations of the effects of FGFR-3 mutations may induce this variability.

CLINICAL REPORTS

Case 1

The affected fetus was the third child of a 23-year-old woman of Turkish origin. In the mother's anamnesis one spontaneous abortion of unknown cause in early pregnancy was mentioned. In addition, she had two healthy children. There were no further reports of skeletal malformations in the family history. During the course of routine ultrasound examination in the 16th and 18th week of gestation, a significant deficiency of limb length was seen along with slight long bone bowing and a very narrow chest. In addition, there was a ventricular septal defect. Based on the sonographic diagnosis of lethal osteochondrodysplasia, the pregnancy was terminated by prostaglandin infusion. The fetus died during labour.

After birth, the fetus presented with symmetrically short limbs with only slight bowing of the long bones (Fig. 1a,b). The trunk appeared normal in length and there were no further significant external abnormalities, particularly of the skull and face. The thorax was narrow and the abdomen was markedly protuberant. Radiographs confirmed the external observations and demonstrated platyspondyly (Fig. 1b). The long bones were only slightly bowed, the metaphyses seemed to be flared with irregular margins and iliac bones were misshapen with triangular formation and trident inferior margins (Fig. 2a). In addition, the ribs were short and the costal osseous transition zone appeared wide (Fig. 1b).

Autopsy showed a ventricular septal defect (0.4 cm in diameter) and bilaterally symmetric hypoplasia of the brain with relative enlargement of the diencephalon and both hippocampuses. Additionally, focal polymicrogyria was seen. Both lungs were reduced in weight and histologically pulmonary maturation was retarded.

Histological examination of the osteochondral junction zone demonstrated normal resting cartilage and a moderate reduction of the chondrocyte proliferative zone and columnar formation. Cartilage vasculature within the canals was unremarkable, and the number of penetrating vascular canals in the growth zone appeared enhanced. However, there was no marginal interposition of fibrous tissue; thus, periosteal ossification did not seem to be enhanced when compared to endochondral bone formation (Fig. 3). Primary trabecular bone was regularly formed and osteoid production seemed unaltered.

SSCP analysis and direct sequencing of the FGFR-3 gene was performed on DNA isolated from cultured chondrocytes. Conditions used for PCR amplification were the same as described previously [Tavormina et al., 1995; Rousseau et al., 1995]. A single base substitution at nucleotide 1118 resulted in the replacement of a tyrosine residue by a cysteine at position 373 of the protein.

Case 2

This pregnancy also was terminated in the 21st week of gestation following the ultrasound diagnosis of a lethal OCD. It was based on the shortness and mild to moderate bowing of limbs, a narrow chest, and protruding abdomen. The mother was a 31-year-old woman of German descent with no family history of skeletal dysplasia. She had previously had two spontaneous abortions, but no further information was available on these gestations.

At autopsy, the fetus presented with short limbs and normal length of the trunk, narrow chest and protruding abdomen. As in case 1, face and skull appeared normal (Fig. 1c,d).

Radiographs confirmed the macroscopic observations and showed platyspondyly (Fig. 1d), significantly bowing of long bones, enlarged metaphyses with irregular margins (Fig. 2b), malformed iliac bones with trident inferior margins, but without characteristic triangular shape. The ribs were short and the osteochondral transition seemed to be irregularly widened (Fig. 1d). Autopsy showed no malformation of viscera, heart or CNS.

The histological findings of the osteochondral zone closely resembled those of case 1. Thus, the resting cartilage zone was unremarkable, while the zones of proliferation and hypertrophy seemed to be moderately reduced (Fig. 4). The number of penetrating vascular canals was increased, while an interposition of fibrous tissue was not detected at the marginal cartilage zone. Ossification was not altered.

Cultured chondrocytes were again used for DNA analysis of the FGFR-3 gene. In this instance a common substitution at nucleotide 742 was identified which converted an arginine residue at position 248 of the protein into a cysteine.

DISCUSSION

Recent molecular analyses have shown that the underlying causes of thanatophoric dysplasia (TD) are mutations in the gene for the fibroblast growth factor receptor-3 [Tavormina et al., 1995; Rousseau et al., 1995]. The two major subtypes of TD, types I and II, appear to be accounted for by mutations in different domains of the receptor. In addition to these two major subtypes of TD, also termed the "curved-bone type" (type I), and the "straight-bone type" (type II), there has been some evidence that there may exist further subtypes of TD, such as the "Glasgow variant" or others [see, e.g., Spranger and Maroteaux, 1990], which, however, have not been characterized at the molecular level. In addition, the delineation of TD from related phenotypes belonging to the group of "platyspondylic OCDs" is often difficult and the elucidation of the molecular defects in the latter group of rare disorders might be helpful for the diagnosis. Therefore, the definition of subtypes of TD and the detection of their underlying molecular defects are essential for the understanding of the phenotypic effects of distinct mutations.

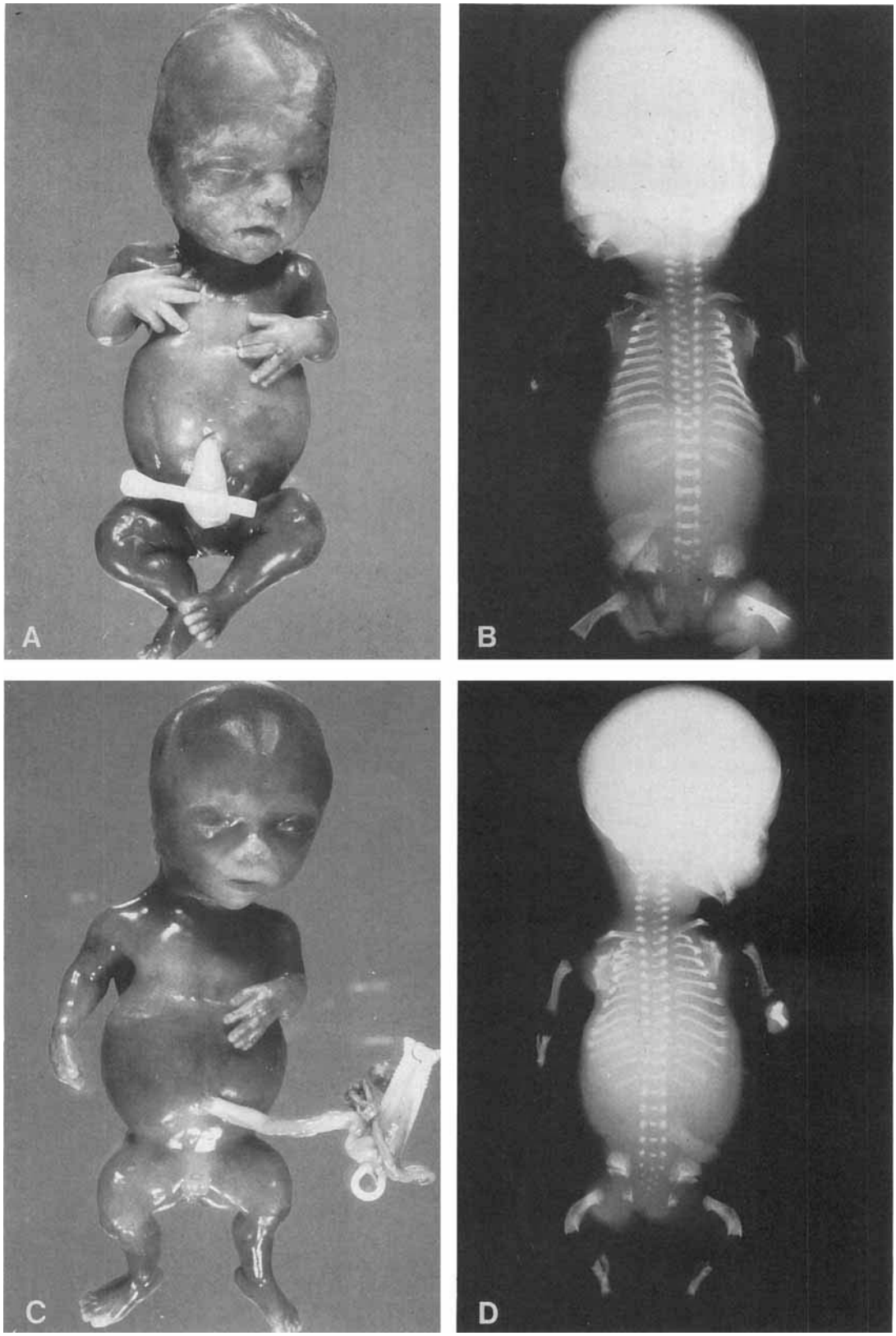

Fig. 1. Macroscopic and radiologic findings in the two cases. **A:** Fetus 1 has short-limbed dwarfism with relatively normal body length. Note the normal face. **B:** The corresponding radiograph of fetus 1 demonstrates severe platyspondyly and malformation of long bones and iliac bones. **C:** Fetus 2 with similar macroscopic changes as case 1 with shortness of all limbs. Note normal face. **D:** The corresponding radiogram of fetus 2 again shows some changes of TD, particularly platyspondyly, curved and short long bones with irregular metaphyses.

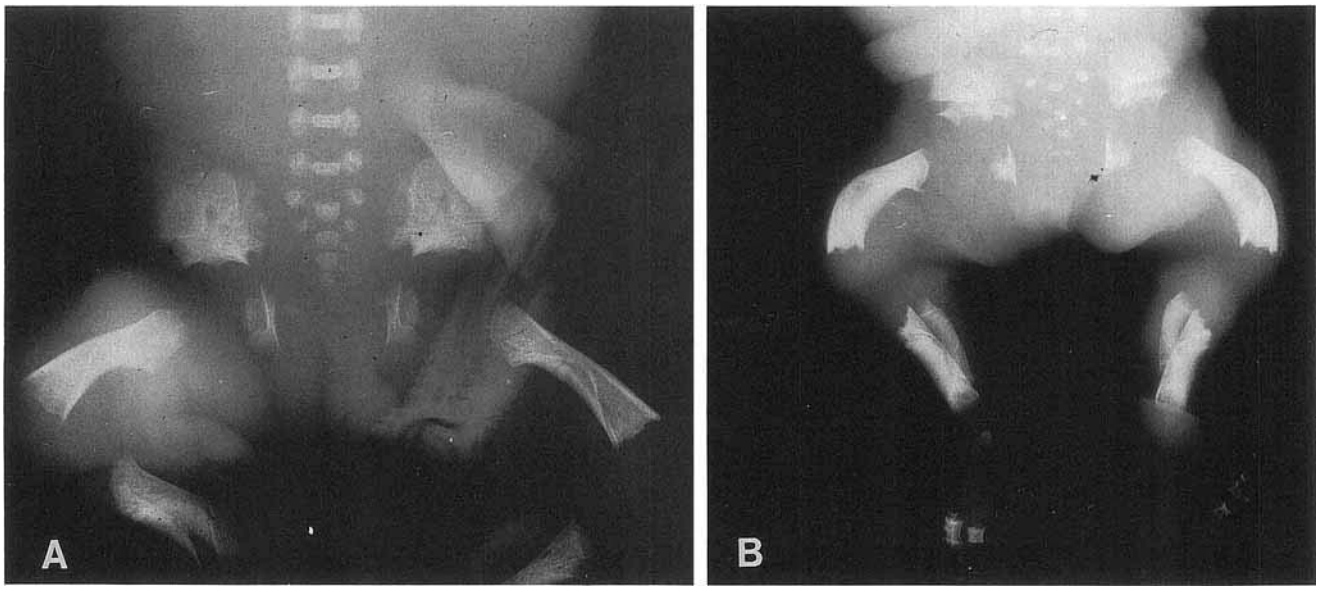

Fig. 2. Radiograms with higher magnifications of the lower limbs of cases 1 **(A)** and 2 **(B)** with limb shortness, metaphyseal irregularities and the "straighter" type of long bones of case 1 when compared to the more "curved" type of long bones in case 2.

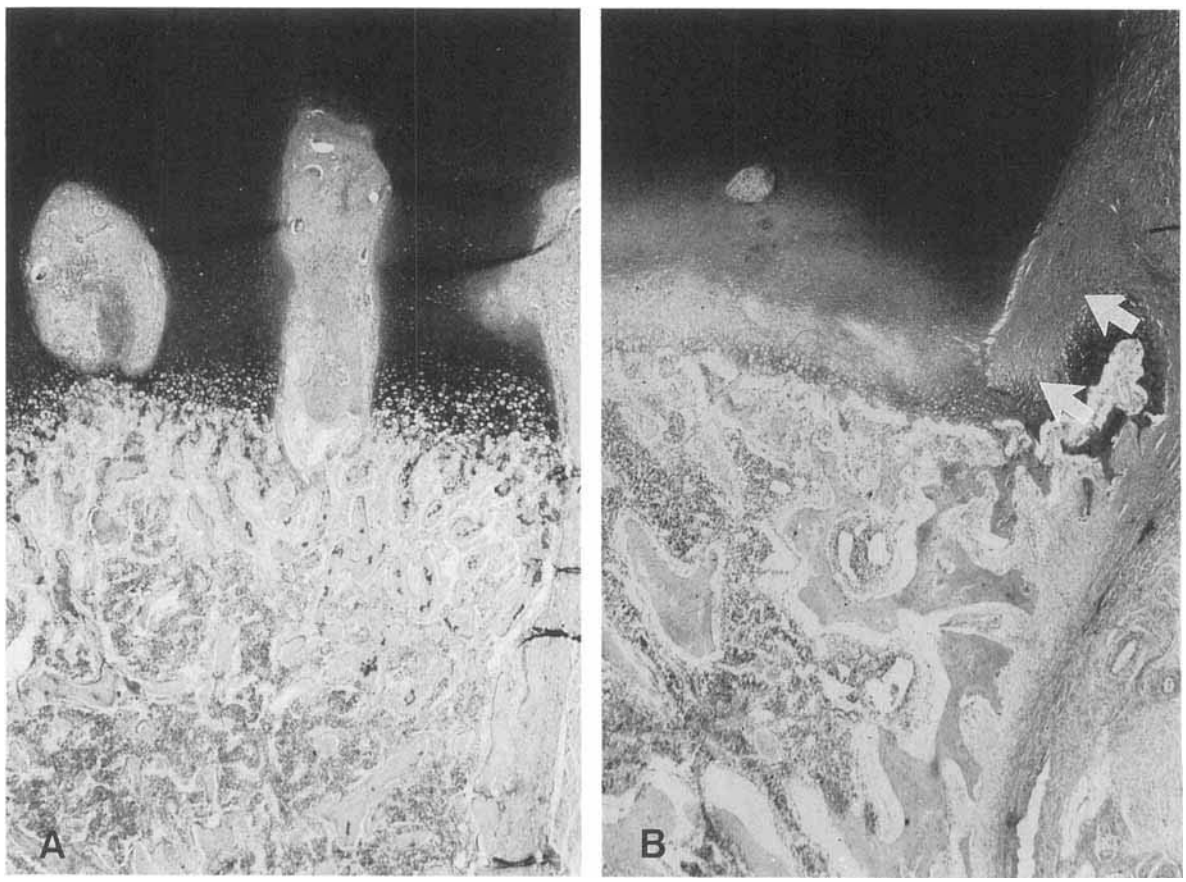

Fig. 3. Histological findings of the osteochondral transition zone in case 1 **(A)** when compared to a control case with TD type I **(B)**. Note in (A) the lack of interposition of a fibrous tissue band at the transition zone to the periosteal ossification (arrows) which is typically present in (B). Note also the enhanced number of penetrating vascular canals in (A). (A,B: alcian-blue staining, ×50).

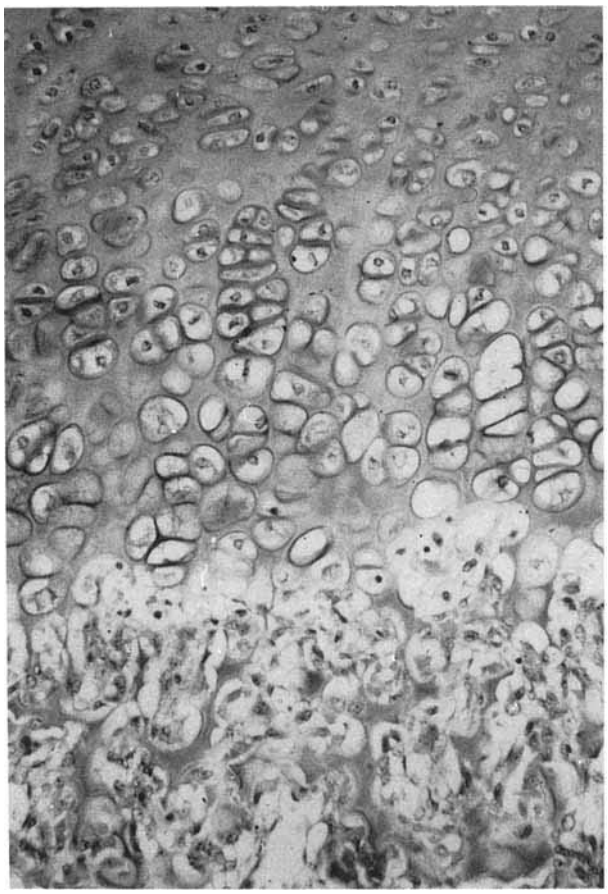

Fig. 4. Micrograph showing the cartilage proliferation and hypertrophic zone in case 2 with only mild reduction of both proliferation and hypertrophy. HE, ×400.

Here, we describe two fetuses at 21 weeks of gestation terminated after ultrasound diagnosis of lethal OCD. Both have typical manifestations of TD, such as severe platyspondyly and malformation of iliac bones, but also distinct differences to the usual types of TD (see Table I). These comprise primarily the lack of facial anomaly and distinct histopathological changes, including a mildly reduced zone of chondrocyte proliferation and hypertrophy and the lack of interposition of a fibrous tissue band at the transition zone between cartilage and the "overgrowing" periosteal ossification, as it is usually seen in TD [Horton et al., 1988]. To the best of our knowledge, these abnormalities have not been yet attributed to TD so that we suggest that these two cases may represent a further subtype of TD which seem to have a milder phenotype of affected individuals.

Table I. Comparison of the Main Findings of the Two Cases in Comparison to TD Types I and II

	TD I	TD II	Case 1	Case 2
Limb shortness	+	+	+	+
Long bone bowing	+	−/(+)	(+)	+
Facial abnormality	+	+	−	−
Platyspondyly	+	+	+	+
Normal resting cartilage	+	+	+	+
Reduced chondrocyte prolif./hypertrophy	+	+	(+)	(+)
Interposed fibrous tissue	+	+	−	−
Enhanced penetrating vascular channels	+	+	+	+

In addition to these common manifestations, there seem to be some interindividual heterogeneity. Thus, our case 1 presented with somewhat "straighter" long bones when compared to case 2 who has typical "curved" bones (see Fig. 2). Similarly, the presence of internal malformations is restricted to case 1, while case 2 is free of internal malformations. The CNS malformations seen in case 1 fit well with those found in other cases of TD, particularly with respect to abnormal gyration and polymicrogyria [Wongmongkoirit et al., 1983; Ho et al., 1984]. The preferential malformation of the CNS may be associated with the high level of expression of the FGFR-3 gene in central nervous tissue [Peters et al., 1993].

In our two cases, the molecular analysis disclosed distinct mutations of the FGFR-3 gene which are identical to those previously found in other cases of TD [Tavormina et al., 1995; Bonaventure et al., submitted for publication]. Thus, other factors than merely the mutation itself appear to modulate the phenotypic expression of the disease.

Recently, we described [Brenner et al., 1993] a different regulation of the clonal growth of human fetal articular and costal chondrocytes under the influence of transforming growth factor-β1 (TGF-β1). These observations clearly indicated that even under normal conditions chondrocytes from various sites, such as articular and costal cartilage, may react differently to defined stimuli, although they are morphologically indistinguishable. Since the FGF-receptor-3 can be regarded as an important mediator of cell stimulation processes, it is conceivable that different "types" of chondrocytes in pathological cartilage may show variable reactivity to external or endogenous stimulation. The mutation may affect these subpopulations of chondrocytes to a different extent, thereby leading to the observed variability of the phenotype. However, the exact nature of such a hypothetically altered stimulation, as well as its possible effects and consequences still remain unclear and deserve further elucidation.

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